Alzheimer's Amyloid Precursor Protein α-Secretase Is Inhibited by Hydroxamic Acid-Based Zinc Metalloprotease Inhibitors: Similarities to the Angiotensin Converting Enzyme Secretase†

S. Parvathy, Ishrut Hussain, Eric H. Karran,‡ Anthony J. Turner, and Nigel M. Hooper*

School of Biochemistry and Molecular Biology, The University of Leeds, Leeds LS2 9JT, U.K., and Neurosciences Research, SmithKline Beecham Pharmaceuticals, Harlow CM19 5AW, U.K.

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ABSTRACT: The 4 kDa β-amyloid peptide that forms the amyloid fibrils in the brain parenchyma of Alzheimer's disease patients is derived from the larger integral membrane protein, the amyloid precursor protein. In the nonamyloidogenic pathway, α-secretase cleaves the amyloid precursor protein within the β-amyloid domain, releasing an extracellular portion and thereby preventing deposition of the intact amyloidogenic peptide. The release of the amyloid precursor protein from both SH-SY5Y and IMR-32 neuronal cells by α-secretase was blocked by batimastat and other related synthetic hydroxamic acid-based zinc metalloprotease inhibitors, but not by the structurally unrelated zinc metalloprotease inhibitors enalaprilat and phosphoramidon. Batimastat inhibited the release of the amyloid precursor protein from both cell lines with an I_{50} value of 3 μM. Removal of the thienothiomethyl substituent adjacent to the hydroxamic acid moiety or the substitution of the P_2' substituent decreased the inhibitory potency of batimastat toward α-secretase. In the SH-SY5Y cells, both the basal and the carbachol-stimulated release of the amyloid precursor protein were blocked by batimastat. In contrast, neither the level of full-length amyloid precursor protein nor its cleavage by β-secretase were inhibited by any of the zinc metalloprotease inhibitors examined. In transfected IMR-32 cells, the release of both the amyloid precursor protein and angiotensin converting enzyme was inhibited by batimastat, marimastat, and BB2116 with I_{50} values in the low micromolar range, while batimastat and BB2116 inhibited the release of both proteins from HUVECs. The profile of inhibition of α-secretase by batimastat and structurally related compounds is identical with that observed with the angiotensin converting enzyme secretase suggesting that the two are closely related zinc metalloproteases.

Alzheimer's disease is a neurodegenerative disorder characterized by the progressive deposition of the 4 kDa β-amyloid peptide (βA4)[1] in extracellular senile plaques. βA4 is a 40−43 amino acid polypeptide derived by proteolytic cleavage of the β-amyloid precursor protein (APP), a type I integral membrane protein (*1*−*3*). Cleavage of APP at the N-terminus of the βA4 peptide by β-secretase and at the C-terminus by one or more γ-secretases constitutes the amyloidogenic pathway for processing of APP. In addition, APP can be processed by α-secretase, which cleaves within the βA4 domain preventing deposition of this intact amyloidogenic peptide. The identification and characterization of the APP secretases is important for the development of therapeutic strategies to control the build up of βA4 in the brain and the subsequent pathological effects of Alzheimer's disease. However, characterization of the secretases that process APP has proved difficult.

α-Secretase cleaves APP within the βA4 domain between Lys16 and Leu17 (*4*), some 12 residues on the extracellular side of the membrane, releasing the large ectodomain of APP (sAPPα) and thereby precluding formation of the βA4 peptide. Although α-secretase has yet to be isolated, this protease appears to be plasma membrane-associated (*5*, *6*), and its activity is upregulated by phorbol esters and other agents that activate protein kinase C (*7*, *8*). Roberts et al. (*6*) described the development of a cell-free assay for α-secretase utilizing a construct of the C-terminal 105 amino acids of APP linked to the C-terminus of alkaline phosphatase. Expression of this construct in human H4 neuroglioma cells resulted in cleavage at the prototypic Lys16-Leu17 α-secretase cleavage site. Of a range of class-specific protease inhibitors examined, only the zinc-chelating agent, 1,10-phenanthroline caused significant inhibition of α-secretase cleavage.

In the present study, we have examined the effect of a number of more selective zinc metalloprotease inhibitors on the processing of APP in two neuronal cell lines. Using site-specific antibodies, we show that α-secretase, but not β-secretase, is inhibited by batimastat, marimastat, and other structurally related hydroxamic acid-based zinc metalloprotease inhibitors. The pattern of inhibition observed resembles

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* To whom correspondence should be addressed. Tel. +44 113 233 3163; Fax. +44 113 233 3167; e-mail: n.m.hooper@leeds.ac.uk.

‡ SmithKline Beecham Pharmaceuticals.

[1] Abbreviations: ACE, angiotensin converting enzyme; ADAM, a disintegrin and metalloprotease-like protein; APP, amyloid precursor protein; βA4, β-amyloid peptide; MTT 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; sAPPα, APP released by α-secretase; sAPPβ, APP released by β-secretase.

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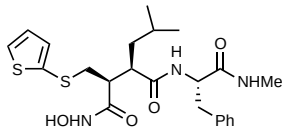
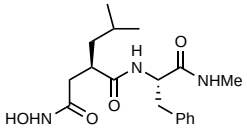
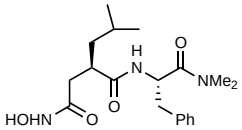
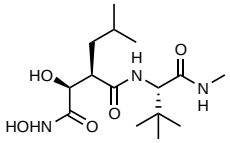
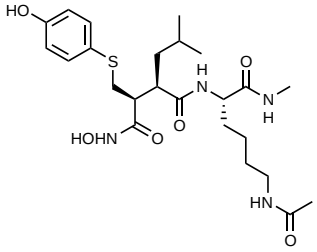
Table 1: Structures and Inhibitory Effects of Compounds on α-Secretase, ACE Secretase, and Collagenase

compd	structure	α-secretase I_{50} μM[a]	ACE secretase I_{50} μM[b]	collagenase I_{50} nM
batimastat		3.3 ± 1.2	1.6 ± 0.5[c]	5[c]
1		17.9	35.0[c]	56[c]
4		>20	>100[c]	220[c]
marimastat		1.2	8.3	5
BB2116		7.7 ± 1.9	3.5 ± 0.8	4

[a] IMR-32 cells were incubated in the absence or presence of the indicated inhibitors as described in the Experimental Section. Media was harvested and sAPPα quantified by densitometric analysis of the immunoelectrophoretic blots as in Figure 2. [b] The effect of the compounds on the activity of ACE secretase was determined using the colocalized porcine kidney microvillar membrane assay system as described in the Experimental Section. [c] Data from ref *9*. Results are the mean ± SEM of three determinations or the mean of duplicate determinations.

that of the angiotensin converting enzyme (ACE) secretase (*9*) and the recently isolated tumor necrosis factor-α convertase (*10*, *11*), suggesting that α-secretase may also be a member of the ADAMs (a disintegrin and metalloprotease-like) family of zinc metalloproteases.

EXPERIMENTAL SECTIONS

Materials. Batimastat (BB94), marimastat (BB2516), and compounds 1 and 4 (*9*) were synthesized at SmithKline Beecham Pharmaceuticals (Harlow, U.K.). Compound 1 differs from batimastat only by the absence of the thienothiomethyl substituent adjacent to the hydroxamic acid moiety, while compound 4 differs from compound 1 by the presence of a secondary amine at its C-terminus (see Table 1 for structures) (*9*). TAPI-2 was a gift from Dr. R. Black (Immunex, Seattle, WA). BB2116 was a gift from Dr. A. Drummond (British Biotechnology Pharmaceuticals, Oxford, U.K.). Antibodies to APP (Ab1−25, Ab1A9, and Ab54) were generated by SmithKline Beecham Pharmaceuticals (Harlow, U.K.) and Ab6E10 was purchased from Senetek (Maryland Heights, MO).

Cell Culture. The neuronal cell lines IMR-32 (*12*) and SH-SY5Y were cultured in Dulbecco's modified Eagle's medium/Ham's F12 supplemented with 10% foetal bovine serum, penicillin (50 units/mL), streptomycin (50 mg/mL), and 2 mM glutamate (all Gibco BRL, Paisley, U.K.). Cells were maintained at 37 °C in 5% CO_2 in air. Carbachol (Sigma Chemical Co., Poole, U.K.) was used at 20 μM for 7 h at 37 °C. HUVECs (human umbilical vein endothelial cells; Clonetics, San Diego, CA) were seeded at 2.5×10^3 cells/cm^2 in Endothelial Cell Basal Medium supplemented with 2% foetal bovine serum, 1 ng/100 mL human recombinant epidermal growth factor, 0.1 mg/100 mL hydrocortisone, 5 mg/100 mL gentamicin, 5 μg/100 mL amphotericin-B, and 1.2 mg/mL of bovine brain extract. When the cells were 70−80% confluent, the media was changed to Opti-MEM and the cells incubated with the indicated inhibitors for 7 h. The medium was then harvested, concentrated and, either subjected to immunoelectrophoretic blot analysis or assayed for ACE activity. For analysis of cell-associated APP, cells were washed with phosphate-buffered saline (20 mM Na_2HPO_4, 2 mM NaH_2PO_4, 150 mM NaCl, pH 7.4) and scraped from the flasks into phosphate-buffered saline. Following centrifugation at 500*g* for 10 min to pellet the cells, the cells were lysed in 0.1 M Tris/HCl, 5 mM EDTA, 1% Triton X-100, pH 7.4, containing leupeptin (1 μg/mL) and dichloroisocoumarin (10 μM).

Cell Viability Assay. Cells were plated at a density of 3×10^4 cells/cm^2 in 0.1 mL of medium/well. After a 24 h incubation, the medium was changed to serum free medium. Different compounds were then added at the indicated concentration. After a further 24 h, 10 μL of 5 mg/mL MTT (3-[4,5-dimethylthiazol-2-yl]2,5-diphenyl tetrazolium bromide) in phosphate-buffered saline was added to each well

and incubated for 4 h at 37 °C in 5% CO_2 (*13*). The reaction was stopped by adding 0.1 mL of solubilization buffer (20% SDS, 50% dimethylformamide, pH 4.7). Absorbance of the dissolved formazan crystals was monitored at 492 nm. Cell viability was monitored by comparing the absorbance reading of cells with medium alone to that of the absorbance reading of cells with the test compounds. With the IMR-32 and the SH-SY5Y cells, all of the compounds at concentrations up to 20 mM gave a cell viability of >92.4%, except for batimastat, which resulted in a cell viability of 82.2%.

Transfection of IMR-32 Cells. The expression vector pECE containing a C-terminal fragment of human ACE in which the N-terminal signal peptide was fused with the C-terminal domain and transmembrane domain (pECE hACE; *14*) was used for transfection of IMR-32 cells. Each 175 cm^2 flask of IMR-32 cells was transfected with 5 μg of DNA and 25 μL of lipofectAmine as cationic lipid in 5 mL of Opti-MEM. After incubation for 3 h at 37 °C, 5% CO_2 fresh Dulbecco's modified Eagle's medium/Ham's F12 supplemented with 10% foetal bovine serum was added (15 mL/flask). Following an overnight incubation, cells were washed with phosphate-buffered saline and then incubated with the indicated inhibitors (all at 20 μM) in Opti-MEM. Medium was recovered after 7 h, the amount of sAPPα determined by immunoelectrophoretic blot analysis, and the amount of ACE determined by enzyme assay.

SDS−PAGE and Immunoelectrophoretic Blot Analysis. Ab1−25 and Ab6E10 both recognize the N-terminal sequence of the βA4 peptide and thus only detect sAPPα. Ab1A9 recognizes the neoepitope formed at the C-terminus of the large ectodomain of APP following β-secretase cleavage (Karran et al., unpublished results). As this neoepitope is cryptic in both full-length APP and sAPPα, Ab1A9 recognizes only sAPPβ. Ab54 recognizes the C-terminal cytosolic domain of APP and was used to detect full-length protein. Samples were resolved on SDS−polyacrylamide gels and blotted onto poly(vinylidene) difluoride membranes (Immobilon P, Millipore) as described previously (*15*). Membranes were probed with antibodies to APP (Ab1−25 at a dilution of 1:4000; Ab1A9 at a dilution of 1:3000; Ab6E10 at a dilution of 1:2000; Ab54 at a dilution of 1:20000) followed by a secondary horseradish peroxidase-conjugated antibody. Bound antibody was detected with the enhanced chemiluminescent detection system (Amersham, Slough, U.K.). Protein concentrations were determined using the bicinchoninic acid method (*16*).

Colocalized Angiotensin Converting Enzyme Secretase Assay. This was performed as described in ref 9. Porcine kidney cortex microvillar membranes were preincubated in 0.1 M borate buffer, pH 8.3, for 20 min at 4 °C in the absence or presence of inhibitors, and then incubated at 37 °C for 4 h. After the incubation, the secretase cleaved form of ACE was separated from the uncleaved membrane-bound form by temperature-induced phase separation in Triton X-114 (*17*). The resulting detergent-rich and aqueous phases were assayed for ACE enzymic activity with BzGly-His-Leu as substrate. Secretase activity is equivalent to the amount of ACE in the final aqueous phase as a percentage of the total amount of ACE in both the aqueous and detergent-rich phases.

Angiotensin Converting Enzyme Assay. ACE enzymic activity was determined with BzGly-His-Leu (5 mM) as substrate in 0.1 M Tris/HCl, 0.3 M NaCl, 10 μM $ZnCl_2$, pH 8.3. Reactions were terminated by heating at 100 °C for 4 min, and the substrate and reaction products resolved and quantified by reverse-phase HPLC as described previously (*15*).

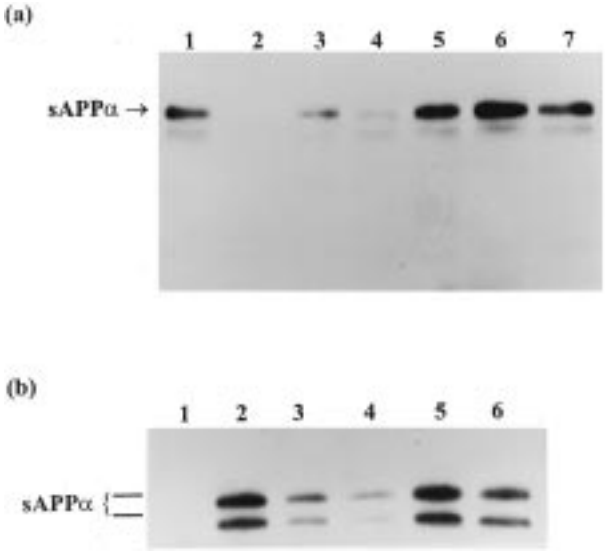


FIGURE 1: Effect of zinc metalloprotease inhibitors on the activity of α-secretase. Cells were incubated in the absence or presence of the indicated compound in the medium. After 7 h, the medium was removed, concentrated, and subjected to immunoelectrophoretic blot analysis with either Ab6E10 (SH-SY5Y cells) or Ab1−25 (IMR-32 cells). (a) IMR-32 cells: lane 1, control (no compound); lane 2, batimastat (20 μM); lane 3, TAPI-2 (20 μM); lane 4, compound 1 (20 μM); lane 5, compound 4 (20 μM); lane 6, phosphoramidon (20 μM); lane 7, enalaprilat (10 μM). (b) SH-SY5Y cells: lane 1, batimastat (20 μM); lane 2, control (no compound); lane 3, compound 4 (20 μM); lane 4, compound 1 (20 μM); lane 5, phosphoramidon (20 μM); lane 6, enalaprilat (10 μM).

RESULTS

The effect of a number of zinc metalloprotease inhibitors on the activity of α-secretase in two neuronal cell lines (IMR-32 and SH-SY5Y) was examined (Figure 1). The release of sAPPα into the medium from the cells was monitored using site-specific antibodies (see Experimental Section). Batimastat at 20 μM completely inhibited the release of sAPPα from both the IMR-32 (Figure 1a, lane 2) and the SH-SY5Y (Figure 1b, lane 1) cells. Batimastat inhibited the release of sAPPα in a dose-dependent manner with an I_{50} of 3.3 ± 1.2 μM (n = 3) for the IMR-32 cells and 3.4 μM (n = 2) for the SH-SY5Y cells (Figure 2). Two structural analogues of batimastat, compounds 1 and 4 (*9*), were also investigated for their effect on the release of sAPPα. Treatment of cells with compound 1 resulted in a partial inhibition of sAPPα release from both the IMR-32 and the SH-SY5Y cells (Figure 1a, lane 4, and Figure 1b, lane 4, respectively), while compound 4 at a concentration of 20 μM had negligible inhibitory effect on the release of sAPPα from the IMR-32 cells (Figure 1a, lane 5) and a slight inhibitory effect on the release of sAPPα from the SH-SY5Y cells (Figure 1b, lane 3). Quantitative densitometric analysis of immunoblots revealed that compound 1 inhibited the release of sAPPα from the IMR-32 cells with an I_{50} of 17.9 μM (Table 1). The related hydroxamic acid-based compounds marimastat and BB2116 also inhibited the release

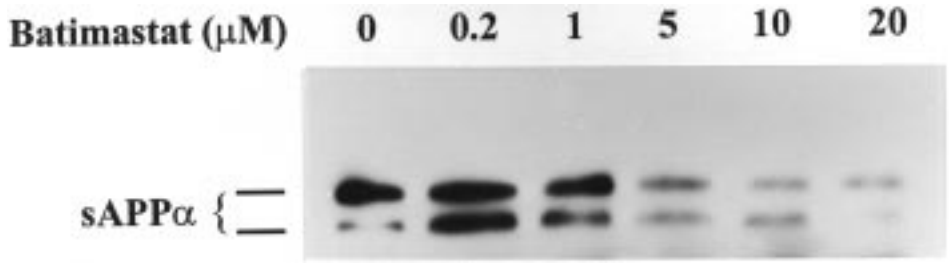


FIGURE 2: Dose-response for the inhibition of α-secretase by batimastat. SH-SY5Y cells were incubated in the presence of the indicated concentration of batimastat for 7 h. The medium was then harvested, concentrated, and subjected to immunoelectrophoretic blot analysis with Ab6E10.

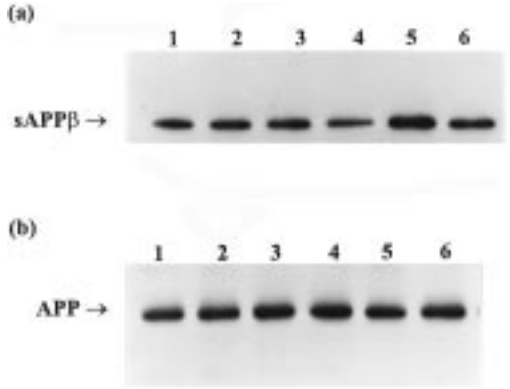


FIGURE 3: Zinc metalloprotease inhibitors have no effect on the activity of β-secretase or on the level of full-length APP. IMR-32 cells were incubated in the absence or presence of the indicated compound in the medium. After 7 h, the medium was removed, concentrated and subjected to immunoelectrophoretic blot analysis with Ab1A9 to detect sAPPβ. The cells were harvested and the cell lysate subjected to immunoeletrophoretic blot analysis with Ab54 to detect full-length APP. (a) sAPPβ: lane 1, control (no compound); lane 2, batimastat (20 μM); lane 3, compound 1 (20 μM); lane 4, compound 4 (20 μM); lane 5, phosphoramidon (20 μM); lane 6, enalaprilat (10 μM). (b) Full-length APP: lane 1, control (no compound); lane 2, batimastat (20 μM); lane 3, compound 1 (20 μM); lane 4, compound 4 (20 μM); lane 5, phosphoramidon (20 μM); lane 6, enalaprilat (10 μM).

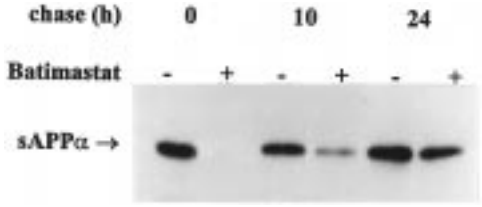


FIGURE 4: The inhibition of α-secretase by batimastat is reversible. IMR-32 cells were incubated in either the presence or absence of 20 μM batimastat. After 7 h, the medium was harvested and replaced with fresh medium not containing batimastat for a further 10 or 24 h. The medium was again harvested, concentrated and subjected to immunoelectrophoretic blot analysis with Ab1−25.

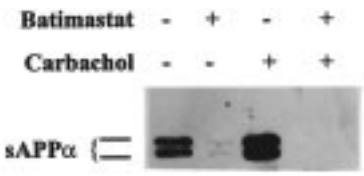


FIGURE 5: Inhibition of the carbachol stimulated release of sAPPα by batimastat. SH-SY5Y cells were incubated in the absence or presence of carbachol and 20 μM batimastat as indicated. After 7 h, the medium was harvested, concentrated and subjected to immunoelectrophoretic blot analysis with Ab6E10.

of sAPPα from the IMR-32 cells with I_{50} values of 1.2 μM and 7.7 μM, respectively (Table 1).

That the inhibitory effect of batimastat is specific for α-secretase is demonstrated by the lack of significant inhibitory effect of batimastat or compounds 1 and 4 on the release of sAPPβ by β-secretase (Figure 3a). The lack of inhibitory effect of the zinc metalloprotease inhibitors on the release of sAPPβ is consistent with the recent observation that β-secretase is inhibited by the serine protease inhibitor 4-(2-aminoethyl)-benzenesulfonyl-fluoride hydrochloride (*18*). In addition, none of the compounds examined altered the level of full-length APP present in the IMR-32 cells as assessed by densitometric analysis of the immunoblots (Figure 3b). In contrast to batimastat, the unrelated zinc metalloprotease inhibitors phosphoramidon (an inhibitor of neprilysin, EC 3.4.24.11) (*19*), and enalaprilat (an inhibitor of angiotensin converting enzyme, EC 3.4.15.1) (*20*) were ineffective in blocking the release of sAPPα from either the IMR-32 or the SH-SY5Y cells (Figure 1). Interestingly, phosphoramidon appeared to cause a slight increase (14 and 19%, respectively) in the production of both sAPPα (Figure 1a, lane 6) and sAPPβ (Figure 3a, lane 5) by the IMR-32 cells as assessed by densitometric analysis of the immunoblots. One possible explanation for this is that phosphoramidon is inhibiting proteases that metabolize the released forms of APP. The effect of phosphoramidon appeared to be cell specific, as no such increase in the production of sAPPα was observed in the SH-SY5Y cells (Figure 1b, lane 5). Previously, phosphoramidon was shown to increase the secretion of βA4 from SH-SY5Y cells but had no effect on the secretion of sAPPα (*21*).

Although none of the compounds were toxic to the cells as assessed with the MTT assay (see Experimental Section), we examined further the toxicity and reversibility of the inhibition of α-secretase by batimastat (Figure 4). IMR-32 cells were incubated in the presence of batimastat for 7 h to block sAPPα release. The medium was then removed and the cells washed extensively with fresh medium before being incubated further in the absence of batimastat. After 10 h, sAPPα could be detected again in the medium, with almost complete recovery being observed after 24 h, indicating that the inhibitory effect of batimastat on the release of sAPPα was reversible and that this compound had no irreversible toxic effect on the cells over the time scale of the experiment.

The effect of batimastat on the carbachol-stimulated release of sAPPα from SH-SY5Y cells was investigated (Figure 5). Incubation of the SH-SY5Y cells in the presence of carbachol led to a significant increase in the release of sAPPα consistent with previous reports (*22*, *23*). When batimastat was present in the medium, no sAPPα could be detected in

Table 2: Inhibition of Both α-Secretase and ACE Secretase in ACE Transfected IMR-32 Cells and HUVECS[a]

cell line	inhibitor	sAPPα (% of control)	ACE activity (% of control)
IMR−32	none	100.0	100.0
	batimastat (20 μM)	12.7	4.8
	marimastat (20 μM)	13.5	8.2
	BB2116 (20 μM)	6.3	7.6
HUVEC	none	100.0	100.0
	batimastat (5 μM)	7.1	6.5
	BB2116 (5 μM)	9.9	14.9

[a] IMR-32 cells were transfected with ACE as described in the Experimental Section. Cells were incubated in the absence or presence of the indicated inhibitors as described in the Experimental Section. Medium was harvested and sAPPα quantified by densitometric analysis of the immunoelectrophoretic blots and ACE quantified by measurement of enzyme activity. Results are representative of two separate experiments.

the medium even upon stimulation of the cells with carbachol (Figure 5).

The effect of batimastat, compounds 1 and 4, marimastat, and BB2116 on the release of ACE from porcine kidney microvillar membranes using the co-localized assay system (*9*) are reported in Table 1. Like batimastat, marimastat and BB2116 inhibited the release of ACE with I_{50} values in the low micromolar range. The effect of batimastat, marimastat, and BB2116 on the activity of both α-secretase and the ACE secretase were directly compared in IMR-32 cells transfected with a cDNA encoding human ACE and in HUVECs which endogenously express both APP and ACE (Table 2). The release of ACE into the medium of the cells was monitored by measuring enzyme activity (see Experimental Section). Batimastat, marimastat, or BB2116 at 20 μM substantially inhibited (>86%) the release of both sAPPα and ACE from the transfected IMR-32 cells, while batimastat and BB2116 at 5 μM substantially inhibited (>85%) the release of the two proteins from the HUVECs.

DISCUSSION

Although the zinc metalloprotease inhibitors enalaprilat and phosphoramidon were ineffective in blocking sAPPα release, we clearly show that batimastat inhibits the action of the APP α-secretase in the neuronal cell lines IMR-32 and SH-SY5Y with an I_{50} of 3 μM. In addition, the release of sAPPα and ACE from HUVECS, which endogenously express both APP and ACE, and from ACE-transfected IMR-32 cells was inhibited substantially by batimastat and BB2116, while marimastat inhibited the release of both sAPPα and ACE from the transfected IMR-32 cells. TAPI-2 also inhibited the α-secretase-mediated release of endogenous APP from the IMR-32 cells. This hydroxamate compound has previously been shown to inhibit the release of APP from chinese hamster ovary cells (*24*), although in that study, the identity of the cleaving enzyme and the site of cleavage were not confirmed. We also examined the effect of structural analogues of batimastat on the activity of α-secretase. Compound 1 differs from batimastat only by the absence of the thienothiomethyl substituent adjacent to the hydroxamic acid moiety (Table 1) and yet is less potent than batimastat in inhibiting α-secretase. Compound 4, which differs from compound 1 by the presence of a secondary amine at its C-terminus, was even less potent. This pattern of inhibition is identical with that recently observed for the secretase involved in releasing ACE from the membrane in a soluble form (Table 1) (*9*). In addition, α-secretase and ACE secretase share a number of other properties in common. Both secretases appear to be integral membrane proteins, resistant to removal from the membrane by high salt, and solubilized by Triton X-100 and 3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate but not by *n*-octyl β-D-glucopyranoside (*6*, *9*, *25*). Furthermore, both secretases cleave their respective substrates between a basic and a hydrophobic residue (Lys-Leu and Arg-Leu) (*4*, *26*) and are stimulated by phorbol esters (*27*, *28*). Thus, it would appear that both α-secretase and ACE secretase are either the same, or closely related, zinc metalloproteases.

Batimastat, marimastat, and BB2116 are all hydroxamic acid-based zinc metalloprotease inhibitors that were originally designed as inhibitors of matrix metalloproteases (*29*, *30*). From studies with the zinc metalloprotease thermolysin, hydroxamates were identified as the preferred zinc ligand. Studies of batimastat and other hydroxamates cocrystallized with the snake venom metalloprotease atrolysin C (EC 3.4.24.42) (*31*, *32*) or the matrix metalloprotease collagenase (EC 3.4.24.34) (*33*, *34*) clearly show that these compounds bind at the active site and coordinate to the essential zinc ion. As batimastat, marimastat, BB2116, compounds 1 and 4 inhibit collagenase with I_{50} values that range from 5 to 220 nM (Table 1), it would appear that marked differences exist between the recognition features essential for the inhibition of α-secretase and the matrix metalloproteases, suggesting that α-secretase is a distinct, but possibly related, zinc metalloprotease. This observation is consistent with the lack of cleavage of full-length APP by gelatinase A (*35*) and the lack of inhibition of sAPPα release by either tissue inhibitor of metalloproteases (TIMP-1) (*36*) or α_2-macroglobulin (*6*).

The secretion of sAPPα is known to be enhanced by phorbol esters and other activators of protein kinase C (*2*). This effect is probably through activation of α-secretase either directly or indirectly via phosphorylation (*7*). The muscarinic agonist carbachol activates protein kinase C through the generation of diacylglycerol and inositol 1,4,5-trisphosphate leading to an increase in sAPPα release (*22*, *23*). In SH-SY5Y cells, carbachol dramatically increased the release of sAPPα, and batimastat completely blocked this enhanced release of APP, indicating that a single activity is probably involved in both the basal and protein kinase C-stimulated release of APP.

Both α-secretase and the recently isolated and cloned tumor-necrosis factor-α convertase (*10*, *11*) are inhibited by batimastat and the related hydroxamic acid-based compounds BB2116 and TAPI-2 with similar potencies. Tumor-necrosis factor-α convertase, a membrane-bound enzyme, belongs to the reprolysin, adamalysin or ADAMs (A Disintegrin And Metallo) family of zinc metalloproteases that are similar to, but distinct from, the matrix metalloproteinases (*37*). Thus, α-secretase, as well as ACE secretase (*9*), may also be members of this expanding family of mammalian membrane-bound zinc metalloproteases. Although it is now apparent that the release of a range of structurally and functionally diverse membrane proteins (including APP, ACE, tumor necrosis factor-α, L-selectin, transforming growth factor-α, interleukin-6 receptor, and tumor necrosis factor receptors I and II) is blocked by hydroxamic acid-based zinc metallo-

protease inhibitors such as batimastat, BB2116, and TAPI-2 (*9*, *24*, *38*), it remains to be determined whether there is a single protease responsible for the cleavage and release of these structurally and functionally unrelated membrane proteins or whether there is a larger family of integral membrane zinc metalloproteases with each member showing a limited cleavage specificity. Already several members of the mammalian ADAMs family have been identified (*39*−*43*), for which, other than the tumor-necrosis factor-α convertase, no physiological substrate has yet been identified. Thus, a major role for ADAMs may be in the selective release of proteins from the cell surface.

Another interesting aspect arising from this study is the use of hydroxamic acid-based metalloprotease inhibitors as potential therapeutics and the need for highly specific inhibitors. Marimastat is currently undergoing assessment in patients as a potential anti metastatic agent (*44*). In light of the present study and other recent reports (*9*−*11*), it may be prudent to assess whether marimastat has secretase inhibitory activity in vivo in addition to its efficacy as a matrix metalloprotease inhibitor. If such inhibitors also are able to inhibit α-secretase in vivo, and are used in chronic therapy, an unwanted side effect may be an increase in the amount of APP that is processed via the amyloidogenic pathway.

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